A Clinical Study Evaluating the Effects of a Sonic Toothbrush with Ultrasound Waveguide in Disrupting Plaque with and without Bristle Contact

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Abstract

Purpose: The purpose of this study was to assess the *in vivo* plaque removal efficacy of the newly marketed sonic/ultrasonic toothbrush. Plaque removal resulting from the toothbrush being held approximately 3 mm from the tooth surface was compared versus a no brushing control. Also, plaque removal resulting from the brush being used according to the manufacturer's instructions was compared versus a control of using the brush (with power turned off) like a manual toothbrush would be used.

Methods: This was a replicate use, four-treatment, examiner-blind, randomized, eight-period crossover design single brushing plaque study involving 31 subjects. The four treatment regimens consisted of (1) brushing for two minutes with the Ultreo powered toothbrush according to manufacturer's instructions, (2) brushing for two minutes with the Ultreo toothbrush (power turned off) using the brush like a manual toothbrush, (3) having a trained dental hygienist hold the Ultreo toothbrush head 3 mm from tooth surfaces for a total of 2 minutes, or (4) swishing with a dentifrice slurry for one minute in the absence of toothbrushing. For each subject, an experienced, calibrated plaque examiner performed the Turesky Modified Quigley-Hein Plaque Index prior to brushing and following brushing. The difference (baseline minus post-regimen) in average scores was calculated for each subject. The difference scores were analyzed for treatment regimen differences using a mixed model analysis of covariance (with baseline whole-mouth average score as the covariate and subjects considered random) for a crossover design.

Results: Adjusted mean plaque removal scores (baseline plaque score minus post-brushing plaque score) were 0.052 for swishing with a dentifrice slurry, 0.058 for the dental hygienist holding the Ultreo toothbrush approximately 3 mm from tooth surfaces, 0.536 for the Ultreo toothbrush used according to manufacturer's instructions and 0.666 for the Ultreo toothbrush (power turned off) used like a manual toothbrush. The difference between the Ultreo toothbrush held approximately 3 mm from tooth surfaces and swishing with a dentifrice slurry was not statistically significant ($p=0.808$).

The adjusted mean plaque removal score for the Ultreo toothbrush (power turned off) used like a manual toothbrush was statistically significantly ($p<0.001$) greater than corresponding score for the Ultreo toothbrush used per manufacturer's instructions. Ultreo used like a manual toothbrush had an adjusted mean plaque removal score that was 12.4% greater than that for Ultreo used per manufacturer's instructions. Finally, plaque removal scores for the Ultreo toothbrush used per manufacturer's instructions and used like a manual toothbrush were statistically significantly ($p<0.001$) greater than plaque removal scores for the non-brushing treatment regimens.

Clinical Significance:

This study supports that the Ultreo power toothbrush is effective in removing plaque, when the toothbrush bristles contact the teeth. In contrast to previously reported *in vitro* data, the data from this clinical study fails to support that Ultreo removes plaque by any means other than mechanical.

Introduction

The mechanism through which toothbrushes remove plaque from teeth in the oral cavity has been widely accepted as mechanical disruption of the surface plaque biofilm. This mechanism is dependent on the toothbrush bristles contacting the tooth surface and physically disrupting the plaque. The development of sonic toothbrushes over the last decade has led to a new mechanistic hypothesis regarding plaque removal that involves plaque biofilm disruption that is mediated through dynamic fluid motion (without the aid of mechanical disruption by the toothbrush bristles) in the interproximal regions between teeth. This hypothesis has been supported through a number of *in vitro* studies which collectively report that sonic toothbrushes disrupt plaque biofilm through fluid dynamics.[1-4] In addition, sonic toothbrushes have been reported to remove significantly more plaque biofilm through dynamic fluid motion compared to rotation-oscillation toothbrushes in *in vitro* biofilm models.[5-7]

In diametric contrast to these *in vitro* studies, multiple clinical studies have demonstrated that rotation-oscillation toothbrushes remove more plaque than sonic toothbrushes. In a pair of clinical studies, the rotation-oscillation Oral-B Triumph[a] was shown to remove statistically significantly more plaque than the sonic Sonicare Elite[b].[8,9] Oral-B Triumph reduced plaque scores by 87% while Sonicare Elite reduced plaque scores by 70%.[9] These results are consistent with those previously observed in three studies for Oral-B Professional Care[a] relative to Sonicare Elite and Sonicare Advanced[b].[10-12] In one of these studies, the Oral-B Professional Care toothbrush reduced plaque by 88% compared to Sonicare Elite toothbrush which reduced plaque by 61%.[10] In each of the three studies, the rotation-oscillation Oral-B Professional Care toothbrush delivered statistically significantly greater plaque reduction as compared to the sonic toothbrush control. These clinical observations are further supported by a recent Cochrane Collaboration systematic review which reported that the body of clinical evidence supports that only rotation-oscillation power toothbrushes are superior to manual toothbrushes for plaque removal.[13] The observation that clinical results are routinely different than the *in vitro* observations

calls into question the clinical relevance of these types of *in vitro* plaque biofilm models. In general, these biofilm removal *in vitro* models have a number of aspects, including an absence of toothpaste and a submerged aqueous environment, that are inconsistent with the clinical environment of the oral cavity.

A new powered toothbrush (Ultreo©) that combines sonic bristle motion with an ultrasonic wave generator has recently entered the toothbrush market. This new toothbrush was reported in *in vitro* research to remove a *Streptococcus mutans* biofilm (grown for 48 hrs on either hydroxyapatite discs 5 mm in diameter or on frosted glass slides with grooves 0.2mm wide and 0.75mm deep).[14] The hydroxyapatite discs were positioned an average of 3 mm from the ultrasound waveguide within a dentifrice slurry, while the surfaces of the grooved slides were directly brushed with the bristle tips within a dentifrice slurry. In both of these *in vitro* cases the Ultreo toothbrush removed some or most of the biofilm in areas where the bristles did not make contact. Since *in vitro* findings may or may not be clinically relevant, the current study used the Turesky modification of the Quigley-Hein[15,16] plaque index to evaluate plaque reduction *in vivo* for the Ultreo toothbrush when used in a manner similar to the hydroxyapatite disc study.

The purpose of this 4-treatment, 8-period crossover clinical study was to assess the *in vivo* plaque removal efficacy of the newly marketed sonic/ultrasonic toothbrush. Plaque removal resulting from the toothbrush being held approximately 3 mm from the tooth surface was compared versus a no brushing control. Also, plaque removal resulting from the brush being used according to the manufacturer's instructions was compared versus a control of using the brush (with power turned off) like a manual toothbrush.

Materials and Methods

Study Overview

This was a replicate use, four-treatment, examiner-blind, randomized, eight-period crossover design. Thirty-one adult subjects, between the ages of 18 and 70, were recruited and enrolled into the study based on study criteria. This study was conducted in accordance with the Investigational Review Board's human study approval system. Subjects reviewed and signed an informed consent prior to enrollment. The four treatment regimens consisted of (1) brushing for two minutes with the Ultreo powered toothbrush according to manufacturer's instructions, (2) brushing for two minutes with the Ultreo toothbrush (power turned off) using the brush like a manual toothbrush, (3) having a trained dental hygienist hold the Ultreo toothbrush head 3 mm from tooth surfaces for a total of 2 minutes, or (4) swishing with a dentifrice slurry (1 part dentifrice: 3 parts water) for one minute in the absence of toothbrushing. Subjects were randomly assigned to one of sixteen treatment sequences (approximately 2 subjects per sequence) according to a computer-generated randomization plan prepared in advance of study execution. Each subject used each treatment regimen twice during the course of the study

Inclusion criteria for the study required that the subject must: give written informed consent and receive a copy; be between the ages of 18 and 70; be in good general health as determined by the Investigator/designee based on a review of the medical history/update; possess a minimum of 16 scorable teeth; agree not to participate in any other clinical study for the duration of this study; agree to delay any elective dentistry, including dental prophylaxis, until study completion; agree to return for the scheduled clinical visits and follow study procedures; refrain from all oral hygiene procedures and for at least 23-25 hours prior to each study visit; and refrain from eating, drinking, chewing gum and smoking for 4 hours prior to each study visit. Exclusion criteria for the study included evidence of: severe periodontal disease, including but not limited to purulent exudates, generalized mobility, and/or severe recession; five (5) or more carious

lesions requiring restorative treatment; active treatment for periodontitis active orthodontic therapy, or removable prosthesis; a pacemaker or any other internal device; and any disease or conditions that could be expected to interfere with examination procedures or the subject safely completing the study. At each visit continuance criteria were assessed. Subjects were to be discontinued from the study or be excluded from the analysis if they: participated in any other clinical study for the duration of this study; received any elective dentistry, including dental prophylaxis, until study completion; did not refrain from brushing their teeth and any other oral hygiene procedures for 23-25 hours prior to their appointment; or did not refrain from eating, drinking, chewing gum and smoking for 4 hours prior to their appointment.

At Visit 1, subjects reviewed and signed an informed consent and received a copy. Subjects that met all study entrance criteria were given a tube of Crest Cavity Protection Gel[a] dentifrice and a marketed Ultreo sonic/ultrasonic toothbrush to be used at home for approximately 7 days prior to the start of Period 1 (treatment phase). Subjects received brushing instructions (per manufacturer's instructions) and brushed for 2 minutes at the site under supervision for their first brushing. Subjects were instructed to use the acclimation products in place of their normal products until 48 hours prior to Visit 2, to brush twice daily according to instructions provided, and to bring the acclimation products with them when they return for Visit 2. Subjects were also instructed to use their normal at-home toothbrush with Crest Cavity Protection dentifrice for the period between 48 hours and 24 hours prior to Visit 2 and during the 2-day to 5-day washout intervals prior to Visits 3 – 9.

The following procedures were utilized for study Visits 2 through 9. Subjects returned to the clinic and continuance criteria were assessed. After subjects swished their mouth with red disclosing solution for one minute, the examiner conducted a plaque examination using the Turesky Modified Quigley-Hein Plaque Index. Subjects then performed their assigned treatment regimen. For the three groups involving brushes, a pea-sized amount of marketed toothpaste (Crest Cavity Protection Gel) was applied to the

brush by the site staff. All treatment regimens were performed under observation in a separate enclosed treatment room that was physically separated from the examination operatory. The examiner was not allowed in or around this treatment room to ensure the integrity of the blinding. After completing the assigned treatment regimen, the subjects swished with disclosing solution again for one minute and then received a second plaque examination. For the Ultreo held 3 mm from the tooth regimen, a trained dental hygienist held the Ultreo toothbrush head using the tips of the orange side bristles to touch the incisal tooth edge and marginal gingiva as a guide. The distance of the waveguide from the tooth surface was determined to be 3 millimeters using the orange side bristles as a guide. The hygienist was trained in advance of the study to follow these instructions when moving the toothbrush head through the mouth.

A single experienced calibrated, plaque examiner performed the Turesky Modified Quigley-Hein Plaque Index for each subject. This examiner has previously demonstrated the ability to differentiate known differences between manual and power toothbrushes. The plaque examination was scored on all 28 teeth (excluding 3rd molars, crowns and surfaces with cervical restorations) on buccal and lingual surfaces for 56 sites. The Turesky Modified Quigley-Hein Index consists of the following scores: 0 = No Plaque;1 = Separate flecks of plaque at the cervical margin; 2 = A thin, continuous band of plaque (up to 1 mm) at the cervical margin; 3 = A band of plaque wider than 1 mm, but covering less than one third of the side of the crown of the tooth; 4 = Plaque covering at least one third, but less than two thirds of the side of the crown of the tooth; 5 = Plaque covering two thirds or more of the side of the crown of the tooth.

Statistical Plan

Sample size was based on the availability of toothbrushes. No sample size calculations were performed. Approximately 32 subjects were to be enrolled into the study. The plaque scores were averaged on a per-subject basis so that each subject at each study visit had a single whole-mouth average score at baseline and another whole-mouth average score following their treatment regimen. The difference (baseline minus post-regimen) in

average scores was calculated for each subject. The difference scores were analyzed for treatment regimen differences using a mixed model analysis of covariance (with terms in the model for subjects, periods, treatments and carryover, baseline whole-mouth average score as the covariate and subjects considered random) for a crossover design. The study used sixteen sequences of treatment assignments in order to balance for carryover effects and ensure that valid treatment comparisons could be performed even in the presence of carryover effects.

The adjusted plaque removal mean (from the analysis of covariance) for the Ultreo toothbrush (power off) with manual brushing technique regimen was first compared to the adjusted mean removal for the dentifrice slurry swish (no brushing) control regimen in order to evaluate the sensitivity of this study to detect plaque removal. Because this comparison was statistically significant ($p<0.001$), then the treatment regimen comparisons described in the study objective were performed. The plaque removal adjusted mean resulting from the toothbrush being held approximately 3 mm from the tooth surface was compared versus the dentifrice slurry swish (no brushing) control. The plaque removal adjusted mean resulting from the brush being used according to the manufacturer's instructions was compared versus the Ultreo toothbrush (power off) with manual brushing technique regimen.

In order to more fully understand the study results, supplemental statistical analyses were performed for buccal tooth surfaces, for lingual tooth surfaces, for maxillary teeth and for mandibular teeth. These analyses used the same methods and procedures described above.

Results

A total of 31 subjects were enrolled in the study and data from all subjects were included in the statistical analysis. Three subjects missed one of the 8 plaque evaluation visits and one subject missed two of these visits. Partial data from these subjects were included in the statistical analysis. One other subject was observed using the power toothbrush improperly (not according to the manufacturer's instructions) at the period 1 visit. The data from this subject for this visit were not included in the statistical analysis. There were 26 females and 5 males, the subjects ranged from 28 to 56 years of age and the mean age was 43.9 years. (Table 1)

Baseline plaque scores averaged between 2.181 and 2.216 prior to using each of the four treatment regimens. (Table 2) The baseline scores did not differ among the regimens (p=0.571). Adjusted mean plaque removal scores (baseline plaque score minus post-brushing plaque score) were 0.052 for swishing with a dentifrice slurry, 0.058 for the dental hygienist holding the Ultreo toothbrush approximately 3 mm from tooth surfaces, 0.536 for the Ultreo toothbrush used according to manufacturer's instructions and 0.666 for the Ultreo toothbrush (power turned off) used like a manual toothbrush. The difference between the Ultreo toothbrush held approximately 3 mm from tooth surfaces and swishing with a dentifrice slurry was not statistically significant (p=0.808).

The adjusted mean plaque removal score for the Ultreo toothbrush (power turned off) used like a manual toothbrush was statistically significantly ($p<0.001$) greater than the corresponding score for the Ultreo toothbrush used per manufacturer's instructions. Ultreo used like a manual toothbrush had an adjusted mean plaque removal score that was 12.4% greater than that for Ultreo used per manufacturer's instructions. Finally, plaque removal scores for the Ultreo toothbrush used per manufacturer's instructions and used like a manual toothbrush were statistically significantly ($p<0.001$) greater than plaque removal scores for the non-brushing treatment regimens. There were no adverse events reported during the study.

Baseline scores for buccal surfaces did not differ among the regimens (p=0.379) (Table 3) The smallest adjusted mean plaque removal score for buccal surfaces was observed in

the swishing with a dentifrice slurry regimen (0.056). The greatest difference was found when the Ultreo toothbrush was used like a manual toothbrush (0.869). The difference between the Ultreo toothbrush held approximately 3 mm from tooth surfaces and swishing with a dentifrice slurry was not statistically significant ($p=0.512$). All other pairwise treatment comparisons were statistically significant (all $p<0.001$).

A similar trend was observed for lingual surfaces (Table 4) There was no statistically significant difference in baseline scores among the regimens ($p=0.654$). Adjusted mean plaque removal scores for the four groups were in the following order, from least effective to most effective: Ultreo held approximately 3mm from tooth surfaces (0.033); swishing with a dentifrice slurry (0.049); Ultreo used according to manufacturer's instructions (0.370); and Ultreo used like a manual toothbrush (0.456). Results when Ultreo was held away from tooth surfaces were not signficantly different from swishing with a dentifrice slurry ($p=0.600$). Statistically significant differences were found for all other pairwise treatment comparisons (all $p\leq0.004$).

Analyses were also performed to evaluate plaque removal on maxillary and mandibular teeth. (Tables 5 and 6) Baseline scores did not differ among the regimens for either analysis ($p\geq0.450$). Of the four groups, Ultreo used like a manual brush showed the greatest adjusted mean plaque removal score for maxillary (0.724) and mandibular teeth (0.606). In both analyses, Ultreo used like a manual brush showed statistically significant greater plaque removal versus using the brush according to manufacturer's instructions (both $p<0.001$). The two regimens showing the least amount of plaque removal on maxillary and mandibular surfaces were the swishing only regimen and the regimen in which Ultreo was held away from tooth surfaces. Results for these two groups did not differ significantly ($p=0.667$ maxillary; $p=0.918$ mandibular).

Discussion

This study supports that the Ultreo power toothbrush is effective in removing plaque, when the toothbrush bristles contact the teeth. The toothbrush with power on and used

per manufacturer's instructions removed statistically significantly more plaque compared to the non-brushing (one minute dentifrice slurry rinse) control treatment. However, interestingly the toothbrush removed statistically significantly more plaque with power turned off and used like a manual toothbrush than it did with power turned on. This observation suggests that any plaque removal benefit provided by the ultrasonic mechanism provides is marginal relative to the mechanical cleaning of the bristles.

In contrast to previously reported *in vitro* data[14], this study does not support that the Ultreo brush removes plaque without the toothbrush bristles contacting the tooth surface. The plaque reduction observed in the no brushing group was virtually identical and not statistically significantly different than the plaque removal observed in the Ultreo toothbrush held 3 mm from tooth group. Both groups exhibited a tiny reduction in plaque, which is attributable to the detergent effect of the toothpaste. In addition, the small directional differences observed in the no brushing group versus the Ultreo toothbrush held 3 mm from tooth group comparison is not consistent between the buccal/lingual subsets or between the maxillary/mandibular subsets. In particular for mandibular teeth (where the most fluid would expect to pool) these regimens were nearly identical (comparison p-value 0.918) with respect to plaque removal. The absence of a clinical plaque effect beyond the bristles is further supported by Ultreo removing statistically significantly more plaque when turned off and used like a manual toothbrush than when used with the power turned on. This finding was consistently observed across all subset analyses. Collectively the data fails to support that Ultreo removes plaque by any means other than mechanical.

Given the artificial nature of the *in vitro* study evaluating the Ultreo brush, it is not surprising that it is a poor predictor of the clinical outcome. As noted in the introduction, multiple clinical studies have demonstrated that rotation-oscillation toothbrushes remove more plaque than sonic toothbrushes, even though sonic toothbrushes have been reported to remove significantly more plaque biofilm through dynamic fluid motion compared to rotation-oscillation toothbrushes in *in vitro* biofilm models.[5-7] In a pair of clinical studies, the rotation-oscillation Oral-B Triumph was shown to remove statistically

significantly more plaque than the sonic Sonicare Elite.[8,9] Importantly, *in vitro* biofilm removal models have a number of aspects that are inconsistent with the clinical environment of the oral cavity. For example, these models invariably involve the formation of an artificial plaque biofilm on an artificial surface. In the *in vitro* study with Ultreo, a monoculture *Streptococcus mutans* biofilm was formed over a 48 hour period on hydroxyapatite chips or glass slides. This is in contrast to the oral cavity where an enamel tooth surface is coated with a salivary pellicle which mediates the formation of a complex plaque biofilm involving 30 to 300 bacterial species.[17] The ability to predict properties of naturally occurring plaque biofilms based on knowledge from single species biofilms is very limited, as single species biofilms lack both the genotypic and spatial heterogeneity of natural plaque biofilms.[18] In addition, the sonic brushing in these models is delivered in a submerged aqueous environment, which is inconsistent with the oral cavity environment. There is routinely no more than 1.07 ± 0.39 ml of resting saliva pooled in the floor of the mouth at any time point because of gravity and the swallow reflex.[19] Finally, many of these models do not include dentifrice, which can have antibacterial/antiplaque activity. All of these variables help explain the paradoxical results that *in vitro* biofilm models deliver relative to clinical plaque removal studies with toothbrushes. As such, *in vitro* plaque biofilm models have proven to be a poor predictor of clinical response when applied to sonic and rotation-oscillation power toothbrushes.

a.Procter & Gamble, Cincinnati, OH USA.

b.Philips Oral Healthcare, Inc., Snoqualmie, WA, USA.

c.Ultreo, Inc. Redmond, WA, USA.

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References

1. Stanford CM, Srikantha R, Wu CD. Efficacy of the Sonicare toothbrush fluid dynamic action on removal of human supragingival plaque. *J Clin Dent* 1997; 8(1 Spec No):10-

14.

2. Stanford CM, Srikantha R, Kirchner HL, Wu CD. Removal of supragingival plaque in an intraoral model by use of the Sonicare toothbrush. *J Int Acad Periodontol* 2000;2:115-119.
3. Wu Yuan CD, Anderson RD, McInnes C. Ability of the Sonicare electronic toothbrush to generate dynamic fluid activity that removes bacteria. *J Clin Dent* 1994; 5:89-93.
4. Hope CK, Wilson M. Effects of dynamic fluid activity from an electric toothbrush on in vitro biofilms. *J Clin Periodontol* 2003; 30:624-629.
5. Hope CK, Wilson M. Comparison of the interproximal plaque removal efficacy of two powered toothbrushes using in vitro oral biofilms. *Am J Dent* 2002; 15 (Spec No):7B-11B.
6. Hope CK, Petrie A, Wilson M. In vitro assessment of the plaque removing ability of hydrodynamic shear forces produced beyond the bristles by 2 electric toothbrushes. *J Periodontol* 2003; 74:1017-1022.
7. Adams H, Winston MT, Heersink J, Buckingham-Meyer KA, Costerton JW, Stoodley P. Development of a laboratory model to assess the removal of biofilm from interproximal spaces by powered toothbrushing. *Am J Dent* 2002; 15 (Spec No):12B-17B.
8. Biesbrock AR, Bartizek RD, Walters PA, Warren PR, Cugini MA, Goyal CR. Clinical evaluations of plaque removal efficacy: An advanced oscillating-rotating power toothbrush versus a sonic toothbrush. *J Clin Dent* 2007; In Press.
9. Sharma N, Qaquish J, Cugini M, Thompson MC, Warren PR. Plaque removal of three power toothbrushes. *J Dent Res* 2006; 85: Abstract 2054.
10. Strate J, Cugini MA, Warren PR, Qaquish JG, Galustians HJ, Sharma NC. A comparison of the plaque removal efficacy of two power toothbrushes: Oral-B Professional care series versus Sonicare Elite. *Int Dent J* 2005; 55:151-156.
11. Rosema NA, Timmerman MF, Piscaer M, Strate J, Warren PR, Van der Velden, Van der Weijden GA. An oscillating/pulsating electric toothbrush versus a high frequency electric toothbrush in the treatment of gingivitis. *J Dent* 2005; 33 (Suppl. 1):29-26.

12. Sharma NC, Goyal CR, Qaquish JG, Cugini MA, Thompson MC, Warren PR. Single use plaque removal efficacy of three power toothbrushes. *J Dent* 2005; 33 (Suppl. 1):11-15.

13. Heanue M, Deacon SA, Deery C, Robinson PG, Walmsley AD, Worthington HV, Shaw WC. Manual versus powered toothbrushing for oral health (Cochrane Review). In: The Cochrane Library, Issue 2, 2003. Oxford: Update Software.

14. Roberts FA, Hacker B, Oswald T, McInnes C. Evaluation of ultrasound as a means to remove Streptococcus mutans biofilm. Retrieved 2007. http://www.ultreo.com/web/home/dental-professionals/clinical-research/detail.aspx?O=5688104f2f384f2ea8be6da4c4fb6f23

15. Quigley, GA, Hein JW. Comparative cleaning efficacy of manual and power brushing. *J Am Dent Assoc* 1962;65:26-29.

16. Turesky S, Gilmore ND, Glickman L. Reduced plaque formation by the chloromethyl analogue of victamine C. *J Periodontol* 1970;41:41-43.

17. Moore WEC, Moore LVH. The bacteria of periodontal diseases. *Periodontol 2000* 1994; 5:66-77.

18. Sissons CH. Artificial plaque biofilm model systems. *Adv Dent Res* 1997; 11:110-126.

19. Lagerlof F, Dawes C. The volume of saliva in the mouth before and after swallowing. *J Dent Res* 1984; 63:618-621.

Table 1: Demographics

	N	Age (mean ± s.d.)	Gender (Percent Female)
Population	**31**	**43.9 ± 6.5**	**81%**

Table 2: Plaque Results: All Surfaces

Treatment Group	N[a]	Baseline Score (Mean ± s.d.)	Baseline minus Post-Brushing Difference (adjusted mean[b] ± s.e.)	Pairwise Comparison p-value[c]
Ultreo Off	61	2.202 ± 0.287	0.666 ± 0.024	<0.001
Ultreo On	60	2.181 ± 0.250	0.536 ± 0.024	
Ultreo 3 mm from Tooth	61	2.216 ± 0.326	0.058 ± 0.024	0.808
No Brushing	60	2.207 ± 0.288	0.052 ± 0.024	

a – Subjects used each treatment twice, so the 31 subjects provided 60-61 values for each treatment which were appropriately considered in the statistical anlaysis.

b - Adjusted means and standard errors from analysis of covariance with baseline score as the covariate.

c – For remaining pairwise comparisons, $p<0.001$.

Table 3: Plaque Results: Buccal Surfaces

Treatment Group	N[a]	Baseline Score (Mean ± s.d.)	Baseline minus Post-Brushing Difference (adjusted mean[b] ± s.e.)	Pairwise Comparison p-value[c]
Ultreo Off	61	2.141 ± 0.414	0.869 ± 0.034	<0.001
Ultreo On	60	2.112 ± 0.383	0.697 ± 0.034	
Ultreo 3 mm from Tooth	61	2.182 ± 0.451	0.080 ± 0.034	0.512
No Brushing	60	2.151 ± 0.412	0.056 ± 0.034	

a – Subjects used each treatment twice, so the 31 subjects provided 60-61 values for each treatment which were appropriately considered in the statistical anlaysis.

b - Adjusted means and standard errors from analysis of covariance with baseline score as the covariate.

c – For remaining pairwise comparisons, $p<0.001$.

Table 4: Plaque Results: Lingual Surfaces

Treatment Group	N[a]	Baseline Score (Mean ± s.d.)	Baseline minus Post-Brushing Difference (adjusted mean[b] ± s.e.)	Pairwise Comparison p-value[c]
Ultreo Off	61	2.267 ± 0.300	0.456 ± 0.029	0.004
Ultreo On	60	2.256 ± 0.291	0.370 ± 0.029	
Ultreo 3 mm from Tooth	61	2.255 ± 0.351	0.033 ± 0.029	0.600
No Brushing	60	2.269 ± 0.317	0.049 ± 0.029	

a – Subjects used each treatment twice, so the 31 subjects provided 60-61 values for each treatment which were appropriately considered in the statistical anlaysis.

b - Adjusted means and standard errors from analysis of covariance with baseline score as the covariate.

c – For remaining pairwise comparisons, $p<0.001$.

Table 5: Plaque Results: Maxillary Teeth

Treatment Group	N[a]	Baseline Score (Mean ± s.d.)	Baseline minus Post-Brushing Difference (adjusted mean[b] ± s.e.)	Pairwise Comparison p-value[c]
Ultreo Off	61	2.092 ± 0.291	0.724 ± 0.027	<0.001
Ultreo On	60	2.084 ± 0.259	0.577 ± 0.027	
Ultreo 3 mm from Tooth	61	2.114 ± 0.325	0.065 ± 0.027	0.667
No Brushing	60	2.112 ± 0.301	0.052 ± 0.027	

a – Subjects used each treatment twice, so the 31 subjects provided 60-61 values for each treatment which were appropriately considered in the statistical anlaysis.

b - Adjusted means and standard errors from analysis of covariance with baseline score as the covariate.

c – For remaining pairwise comparisons, $p<0.001$.

Table 6: Plaque Results: Mandibular Teeth

Treatment Group	N[a]	Baseline Score (Mean ± s.d.)	Baseline minus Post-Brushing Difference (adjusted mean[b] ± s.e.)	Pairwise Comparison p-value[c]
Ultreo Off	61	2.317 ± 0.329	0.606 ± 0.030	<0.001
Ultreo On	60	2.285 ± 0.287	0.495 ± 0.030	
Ultreo 3 mm from Tooth	61	2.326 ± 0.377	0.049 ± 0.030	0.918
No Brushing	60	2.310 ± 0.329	0.052 ± 0.030	

a – Subjects used each treatment twice, so the 31 subjects provided 60-61 values for each treatment which were appropriately considered in the statistical anlaysis.

b - Adjusted means and standard errors from analysis of covariance with baseline score as the covariate.

c – For remaining pairwise comparisons, $p<0.001$.

EXECUTIVE SUMMARY

A Study to Assess Plaque Removal Efficacy of a Newly Marketed Ultrasonic Toothbrush Study 2007089

Background and Objective

The clinical plaque removal efficacy of powered toothbrushes using rotation-oscillation motion and sonic motion of the bristles has been widely studied. A new powered toothbrush (Ultreo™) that combines sonic bristle motion with an ultrasonic wave generator has entered the toothbrush market. This new toothbrush was shown in *in vitro* research to remove a *Streptococcus mutans* biofilm (grown for 48 hrs on either hydroxyapatite discs 5 mm in diameter or on frosted glass slides with grooves 0.2mm wide and 0.75mm deep). The hydroxyapatite discs were positioned an average of 3 mm from the ultrasound waveguide within a dentifrice slurry, while the surfaces of the grooved slides were directly brushed with the bristle tips within a dentifrice slurry. In both of these *in vitro* cases the Ultreo toothbrush removed some or most of the biofilm in areas where the bristles did not make contact. Since *in vitro* findings may or may not be clinically relevant, the current study used the Turesky modification of the Quigley-Hein plaque index to evaluate plaque reduction *in vivo* for the Ultreo toothbrush when used in a manner similar to the hydroxyapatite disc study. The purpose of this study was to assess the plaque removal efficacy of the newly marketed sonic/ultrasonic toothbrush. Plaque removal resulting from the toothbrush being held approximately 3 mm from the tooth surface was compared versus a no brushing control. Also, plaque removal resulting from the brush being used according to the manufacturer's instructions was compared versus a control of using the brush (with power turned off) like a manual toothbrush would be used.

Study Design

Study 2007089 employed a 4-treatment, examiner-blind, randomized, 8-period crossover design. The four treatment regimens were: 1) swishing for 1 minute with a dentifrice slurry (no brushing); 2) Ultreo brushing teeth for 2 minutes per manufacturer's instructions; 3) Ultreo held approximately 3 mm from tooth surfaces for 2 minutes by a dental hygienist and 4) Ultreo (power turned off) using the brush like a manual toothbrush for 2 minutes. The subjects were asked to refrain from all oral hygiene procedures for 23 to 25 hours prior to their appointment time. In addition, the subjects were asked to refrain from eating, drinking or smoking for 4 hours prior to their appointment time. Subjects were exposed to each of the 4 treatment regimens twice over the course of the 8 study periods.

At study visit 1, subjects completed an informed consent form and were reviewed for study inclusion/exclusion criteria. Demographic and medical history data were collected. Subjects that met all study entrance criteria were given a tube of Crest Cavity Protection dentifrice and a marketed Ultreo sonic/ultrasonic toothbrush to be used at home for approximately 7 days prior to the start of Period 1. Subjects received brushing instructions (per manufacturer's instructions) and brushed for 2 minutes at the site under supervision for their first brushing. Subjects were instructed to use the acclimation products in place of their normal products until 48 hours prior to Visit 2, to brush twice daily according to instructions provided, and to bring the acclimation products with them when they return for Visit 2. Subjects were instructed to use their normal at-home toothbrush with Crest Cavity Protection dentifrice for the period between 48 hours and 24 hours prior to Visit 2.

At Visit 2, subjects returned to the clinic and continuance criteria were assessed. After subjects swished their mouth with red disclosing solution for one minute, the examiner conducted a plaque examination using the Turesky Modified Quigley-Hein Plaque Index. Subjects then received their assigned treatment regimen for 2 minutes. Depending on the regimen, they either: (1) brushed for

two minutes with the Ultreo powered toothbrush according to manufacturer's instructions, (2) brushed for two minutes with the Ultreo toothbrush (power turned off) using the brush like a manual toothbrush, (3) had a dental hygienist hold the Ultreo toothbrush head approximately 3 mm from tooth surfaces for a total of 2 minutes, or (4) swished their mouth with a dentifrice slurry for 1 minute then did nothing for 1 minute. For regimens (1), (2) and (3), a pea-sized amount of marketed toothpaste was applied to the brush by the site staff. All treatment regimens were performed under observation and with the subjects unaided by a mirror. After completing the assigned treatment regimen, the subjects swished with disclosing solution again for one minute and then received a second plaque examination. Subjects were rescheduled. At visits 3 through 9 the same disclosing, brushing and plaque grading procedure was followed. Subject accountability was documented at the final visit.

A mixed model analysis of covariance (ANCOVA) for a crossover design was applied to the baseline minus post-treatment differences in average whole-mouth plaque scores (with the baseline plaque score as the covariate) in order to assess treatment effects. Tests for carryover effects were also performed for each variable. Adjusted means from the analysis of covariance were compared using appropriate t-tests. All comparisons were two-sided at the 0.05 level of significance.

Results

A total of 31 subjects were enrolled in the study and data from all subjects were included in the statistical analysis. Three subjects missed one of the 8 plaque evaluation visits and one subject missed two of these visits. Partial data from these subjects were included in the statistical analysis. One other subject was observed using the power toothbrush improperly (not according to the manufacturer's instructions) at the period 1 visit. The data from this subject for this visit were not included in the statistical analysis. There were 26 females and 5 males, the subjects ranged from 28 to 56 years of age and the mean age was 43.9 years.

Efficacy

Baseline plaque scores averaged between 2.181 and 2.216 prior to using each of the four treatment regimens. The baseline scores did not differ among the regimens ($p=0.571$). Adjusted mean plaque removal scores (baseline plaque score minus post-brushing plaque score) were 0.052 for swishing with a dentifrice slurry, 0.058 for the dental hygienist holding the Ultreo toothbrush held approximately 3 mm from tooth surfaces, 0.536 for the Ultreo toothbrush used according to manufacturer's instructions and 0.666 for the Ultreo toothbrush (power turned off) used like a manual toothbrush. The difference between the Ultreo toothbrush held approximately 3 mm from tooth surfaces and swishing with a dentifrice slurry was not statistically significant ($p=0.808$).

The adjusted mean plaque removal score for the Ultreo toothbrush (power turned off) used like a manual toothbrush was statistically significantly ($p<0.001$) greater than corresponding score for the Ultreo toothbrush used per manufacturer's instructions. Ultreo used like a manual toothbrush had an adjusted mean plaque removal score that was 12.4% greater than that for Ultreo used per manufacturer's instructions.

Finally, plaque removal scores for the Ultreo toothbrush used per manufacturer's instructions and used like a manual toothbrush were statistically significantly ($p<0.001$) greater than plaque removal scores for the non-brushing treatment regimens.

Safety

There were no adverse events reported during the study.

Summary

- Based on data from the Turesky Modified Quigley-Hein plaque examiner, the Ultreo toothbrush (power turned off) used like a manual toothbrush had statistically significantly greater plaque removal efficacy than the Ultreo toothbrush used per manufacturer's instructions following 2 minutes of brushing ($p<0.001$), with 12.4% higher plaque removal scores than the Ultreo toothbrush used per manufacturer's instructions.

- The difference in plaque removal efficacy between the Ultreo toothbrush held approximately 3 mm from tooth surfaces for 2 minutes and swishing with a dentifrice slurry for 1 minute was not statistically significant (p=0.808)..

- Plaque removal scores for the Ultreo toothbrush used per manufacturer's instructions and used like a manual toothbrush were statistically significantly ($p<0.001$) greater than plaque removal scores for the non-brushing treatment regimens.

- All treatment regimens were well tolerated.

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Clinical Scientist

RD Bartizek
Statistician

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Clinical Data Manager

The Procter & Gamble Company
Cincinnati, Ohio USA

A Study to Assess Plaque Removal Efficacy of a Newly Marketed Ultrasonic Toothbrush

12 July 2007

Protocol Number 2007089

Sponsor:	The Procter & Gamble Company Regulatory and Clinical Development 8700 Mason-Montgomery Road Mason, OH 45040
Principal Investigator	Aaron Biesbrock, DMD, PhD The Procter & Gamble Company Oral Health Science Center – Gwynne Building 602 Main Street Cincinnati, OH 45202

Investigator's Agreement Statement:

I have read, I understand, and I will conduct the study according to this Protocol and Good Clinical Practices.

12 July 07
7/12/07
(D-Mon-Yr)

Signatures below indicate approval of the Protocol.

Medical Monitor/Clinical Scientist:	Name: Aaron Biesbrock, DMD, PhD Phone: 513-622-0316 Fax: 513-622-0560		12 July 07 (D-Mon-Yr)
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Introduction

The clinical plaque removal efficacy of powered toothbrushes using rotation-oscillation motion and sonic motion of the bristles has beed widely studied. A new powered toothbrush (Ultreo™) that combines sonic bristle motion with an ultrasonic wave generator has entered the toothbrush market. This new toothbrush was shown in *in vitro* research to remove a *Streptococcus mutans* biofilm (grown for 48 hrs on either hydroxyapatite discs 5 mm in diameter or on frosted glass slides with grooves 0.2mm wide and 0.75mm deep). The hydroxyapatite discs were positioned an average of 3 mm from the ultrasound waveguide within a dentifrice slurry, while the surfaces of the grooved slides were directly brushed with the bristle tips within a dentifrice slurry. In both of these in vitro cases the Ultreo™ toothbrush removed some or most of the biofilm in areas where the bristles did not make contact. Since *in vitro* findings may or may not be clinically relevant, the current study will use the Turesky modification of the Quigley-Hein plaque index to evaluate plaque reduction *in vivo* for the Ultreo™ toothbrush when used in a manner similar to the hydroxyapatite disc study. The combined sonic/ultrasonic plaque removal effect will also be explored versus an unpowered manual brushing technique.

2. Study Objective(s)

The purpose of this study is to assess the plaque removal efficacy of a newly marketed sonic/ultrasonic toothbrush. Plaque removal resulting from the toothbrush being held approximately 3 mm from the tooth surface will be compared versus a no brushing control. Also, plaque removal resulting from the brush being used according to the manufacturer's instructions will be compared versus a control of using the brush (with power turned off) like a manual toothbrush would be used.

3. Identity of Investigational Product(s)

The table below identifies the products to be tested in this study.

INVESTIGATIONAL PRODUCT*
Ultreo Ultrasonic Toothbrush
All groups will use Crest Regular Cool Mint Gel Dentifrice

4. Overall Study Design and Plan — Description

This is a replicate use, four treatment, examiner-blind, randomized, eight period crossover design. The four treatment regimens consist of 1) no brushing but rinsing with a dentifrice slurry for one minute; 2) Ultreo™ brushing teeth per manufacturer's instructions; 3) Ultreo™ held 3 mm from tooth surface by a dental hygienist and 4) Ultreo™ brush without power using the brush like a manual toothbrush. Each subject will use each treatment regimen twice during the course of the study and each treatment regimer will have a duration of two minutes. Plaque will be scored using the Turesky Modified Quigley-Hein Plaq Index by an experienced examiner. Approximately 32 adult subjects, between the ages of 18 and 70, wil be enrolled into the study based on study criteria. Each subject will use the toothbrush according to manufacturer's instructions for 3-7 days during an acclimation period. The subjects will be asked to refr from all oral hygiene procedures and chewing gum for 23-25 hours prior to each period appointment tim In addition, the subjects will be asked to refrain from eating, drinking, or smoking for 4 hours prior to thei appointment time.

Table 1. Study Schedule by Procedure Type and Visit

Procedures	Accl. Visit 1	P1 Visit 2*	Wash Out Btwn each period	P2 Visit 3	P3 Visit 4	P4 Visit 5	P5 Visit 6	P6 Visit 7	P7 Visit 8	P8 Visit 9
Informed Consent	X									
Health History	X									
Demographics	X									
Inclusion/Exclusion	X									
Continuance Criteria		X		X	X	X	X	X	X	X
Pre-Post Plaque Index		X		X	X	X	X	X	X	X
Adverse Events		X		X	X	X	X	X	X	X
Product Distribution	X	X		X	X	X	X	X	X	X
General Comments		X		X	X	X	X	X	X	X
Subject Accountability				X	X	X	X	X	X	X

Study Procedures:

Acclimation (Visit 1):

Subjects will review and sign an informed consent and receive a copy. Medical history, demographic information, inclusion/exclusion criteria will be collected. Subjects that meet all study entrance criteria will be given a tube of Crest Cavity Protection dentifrice and a marketed Ultreo sonic/ultrasonic toothbrush to be used at home for 3-7 days prior to the start of Period 1. Subjects will receive brushing instructions (per manufacturers instructions) and will brush for 2 minutes at the site under supervision for their first brushing. Subjects will be instructed to use the acclimation products in place of their normal products until 48 hours prior to Visit 2, to brush twice daily according to instructions provided, and to bring the acclimation products with them when they return for Visit 2. Subjects will be instructed to use the normal at-home toothbrush with Crest Cavity Protection dentifrice for the period between 48 hours and 24 hours prior to Visit 2.

Periods 1 - 7: (Visits 2 - 8)

Subjects will return to the clinic and continuance criteria will be assessed. After subjects swish their mouth with red disclosing solution for one minute, the examiner will conduct a plaque examination using the Turesky Modified Quigley-Hein Plaque Index. Subjects will then undergo their assigned treatment regimen for 2 minutes. Depending on the regimen, they will either: (1) brush for two minutes with the Ultreo powered toothbrush according to manufacturer's instructions, (2) brush for two minutes with the Ultreo toothbrush (power turned off) using the brush like a manual toothbrush, (3) have a dental hygienist hold the Ultreo toothbrush head 3 mm from tooth surfaces for a total of 2 minutes, or (4) rinse with a toothpaste slurry for 1 minute. For regimens (1), (2) and (3), a pea-sized amount of marketed toothpaste will be applied to the brush by the site staff. All treatment regimens will be performed under observation and with the subjects unaided by a mirror. After completing the assigned treatment regimen, the subjects will swish with disclosing solution again for one minute and then receive a second plaque examination. Subjects will be rescheduled.

Period 8 (Visit 9):

Subjects will return to the clinic and continuance criteria will be assessed. Following the same procedures addressed in Periods 1 - 7, subjects will swish with red disclosing solution for one minute and then receive a plaque examination by the examiner. They will then undergo their assigned treatment regimen for 1 or 2 minutes (as described above). After completing the assigned treatment regimen, the subjects will swish with disclosing solution again for one minute and then receive a second plaque examination. Subject accountability will be documented at this final visit.

5. Determination of Sample Size

Sample size was based on the availability of toothbrushes. No sample size calculations were performed. Approximately 32 subjects will be enrolled into the study.

6. Blinding, Labeling, and Shipping Plan

All products (n=32) will be packed into individual kit boxes. Subjects will take home the marketed Ultreo Ultrasonic toothbrush with one tube of marketed toothpaste to use during acclimation. In addition, 2 Ultreo ultrasonic toothbrushes and 20 tubes of Crest Cavity Protection Cool Mint Gel (4.6 oz.) will be packaged in a shipper with a contents statement on the outside for use at the clinical site only. Digital timers (2), dosing cups (800) will be supplied to the site as ancillary supplies.

7. Inclusion Criteria

In order to be included in the study, each subject must:

- give written informed consent and receive a copy
- be between the ages of 18 and 70
- be in good general health as determined by the Investigator/designee based on a review of the medical history/update
- possess a minimum of 16 scorable teeth
- agree not to participate in any other clinical study for the duration of this study
- agree to delay any elective dentistry, including dental prophylaxis, until study completion
- agree to return for the scheduled clinical visits and follow study procedures
- refrain from all oral hygiene procedures and chewing gum for at least 23-25 hours prior to each study visit,
- refrain from eating, drinking, chewing gum and smoking for 4 hours prior to each study visit

8. Exclusion Criteria

Subjects are excluded from study participation where there is evidence of:

- severe periodontal disease, including but not limited to purulent exudates, generalized mobility, and/or severe recession
- five (5) or more carious lesions requiring restorative treatment
- active treatment for periodontitis
- active orthodontic therapy, or removable prosthesis,
- a pacemaker or any other internal device,
- any disease or conditions that could be expected to interfere with examination procedures or the subject safely completing the study

9. Continuance Criteria

Subjects may be discontinued from the study or be excluded from the analysis if they:

- participate in any other clinical study for the duration of this study
- receive any elective dentistry, including dental prophylaxis, until study completion
- do not refrain from brushing their teeth and any other oral hygiene procedures for 23-25 hours prior to their appointment
- do not refrain from eating, drinking, chewing gum and smoking for 4 hours prior to their appointment

10. Treatment Compliance

Subjects will be instructed to brush their teeth for 2 minutes under observation unaided by a mirror with their assigned toothbrush and a marketed dentifrice. Subjects will use their own oral care products at home between visits 2 - 9.

11. Efficacy and Safety Variables

For each subject, the plaque examiner will perform the Turesky Modified Quigley-Hein Plaque Index.

A. Turesky Plaque Examination:

The plaque examination will be scored on all 28 teeth (excluding 3rd molars, crowns and surfaces with cervical restorations) on buccal and lingual surfaces for 56 sites.

Turesky Modified Quigley-Hein Index:

0 = No Plaque
1 = Separate flecks of plaque at the cervical margin.
2 = A thin, continuous band of plaque (up to 1 mm) at the cervical margin.
3 = A band of plaque wider than 1 mm, but covering less than one third of the side of the crown of the tooth.
4 = Plaque covering at least one third, but less than two thirds of the side of the crown of the tooth.
5 = Plaque covering two thirds or more of the side of the crown of the tooth.

12. Statistical and Analytical Plans

The plaque scores will be averaged on a per-subject basis so that each subject will have a single whole-mouth average score at baseline and another whole-mouth average score following brushing. The difference (baseline minus post-brushing) in average scores will be calculated for each subject. The difference scores will be analyzed for treatment regimen differences using an analysis of covariance (with baseline whole-mouth average score as the covariate) for a crossover design. Confidence limits on treatment regimen differences will be calculated.

The Ultreo toothbrush (power off) with manual brushing technique regimen will first be compared with the no brushing control regimen in order to evaluate the sensitivity of this study to detect plaque removal. If this comparison is statistically significant ($p<0.05$), then the treatment regimen comparisons described in the study objective will be performed. Plaque removal resulting from the toothbrush being held 3 mm from the tooth surface will be compared versus the no brushing/slurry rinsing control. Plaque removal resulting from the brush being used according to the manufacturer's instructions will be compared versus the Ultreo toothbrush (power off) with manual brushing technique regimen.

Additional statistical techniques may also be applied in order to more fully understand the data.

13. Method of Assigning Subjects to Treatment Groups

Subjects will be randomly assigned to one of the following sixteen treatment sequences (approximately 2 subjects per sequence) according to a computer-generated randomization plan prepared in advance of study execution.

TREATMENT SEQUENCE	TARGET SAMPLE SIZE
ADCBBCDA	2 subjects
BADCCDAB	2 subjects
CBADDABC	2 subjects
DCBAABCD	2 subjects
ACCABBDD	2 subjects
BDDBCCAA	2 subjects
CAACDDBB	2 subjects
DBBDAACC	2 subjects
ABCDBADC	2 subjects
BCDACBAD	2 subjects
CDABDCBA	2 subjects
DABCADCB	2 subjects
AACCBDDB	2 subjects
BBDDCAAC	2 subjects
CCAADBBD	2 subjects
DDBBACCA	2 subjects

Treatment A: Marketed Ultreo powered toothbrush with Crest Cavity Protection Dentifrice (used per manufacturers instructions)
Treatment B: Marketed Ultreo powered toothbrush with Crest Cavity Protection Dentifrice applied 3mm from tooth surface by a dental hygienist.
Treatment C: Marketed Ultreo powered toothbrush with Crest Cavity Protection Dentifrice (power off using the brush like a manual toothbrush)
Treatment D: No brushing (control)

Appendix 1:

ADVERSE EVENT REPORTING

A serious event is defined as an event, which suggests a definite hazard or handicap to the subjects. Serious events are any events resulting in death, life threatening situation, permanent disability, hospitalization or prolonged hospitalization, or congenital anomaly.

When an Investigator is notified of a serious AE, the Investigator must promptly (within 24 hours) notify Procter & Gamble (P&G, the Clinical Trial Manager or the Medical Monitor) of the serious or unexpected event, regardless of causality. Within 5 working days, a written report describing the circumstances of the event must be submitted to P&G. Any AEs (serious or non-serious) continuing at study end must be followed up to resolution unless documented as "not clinically significant" or the subject is lost to follow-up by the Investigator.

ADVERTISING

Any advertisements used in recruitment of subjects must receive prior approval from P&G.

DATA COLLECTION AND RECORD RETENTION

All subject data are collected and reported according to the Protocol, and maintained in a secure area. All study documentation and case report forms (CRFs) must be made available to P&G, and upon request, to relevant authorities for verification/audit/inspection purposes. Completed CRFs, associated source documentation, and study files are maintained by the Investigator for a period of 2 years to 5 years. In the event the Investigator or the Institution cannot meet this obligation, P&G should be contacted as to the disposal or return of these documents.

PROTOCOL AMENDMENTS/CHANGES

Changes to the Protocol affecting the safety of subjects, scope/objectives of the investigation, or the scientific quality of the study are documented as amendments. Such changes receive P&G, Investigator, unless immediate action is required to safeguard subject safety. Administrative/minor changes (e.g., typos, changes in P&G personnel [excluding medical monitor], etc.) are documented as revisions. Any change in P&G's monitoring staff, Clinical Trial Manager or Medical Monitor during the conduct of the study, must be reported to the Investigator.

GOOD CLINICAL PRACTICES

This study is conducted in compliance with applicable sections of the US Federal Regulations governing informed consent (21 CFR 50), IRBs (21 CFR 56), study conduct (21 CFR 312) and the International Conference on Harmonization's Good Clinical Practice Consolidated Guidelines, [ICH-GCPs, as published by the FDA on 9 May 1997, Federal Register, Volume 62, Number 90 pages 25691-25709]). During the course of the trial, the clinical site is monitored by P&G staff (CRA) to ensure compliance with the Protocol, regulations and guidelines, adequacy of the equipment and facilities , and satisfactory data collection.

INSTITUTIONAL REVIEW

This study is not required to receive institutional review based on safety criteria established by P&G.

OBLIGATION OF THE INVESTIGATOR

Following completion of the study, the Investigator shall submit a final report within 30 days to P&G describing the conduct of the study, deviations from planned conduct, early withdrawals and subject accountability, adverse events, and other information on study conduct necessary for full interpretation of collected data.

STUDY MEDICATION DISPENSING, STORAGE AND ACCOUNTING

Test products are stored in a secure area, under environmental condition as required by label instructions or as described in the Protocol, and dispensed only under the authorization of the Investigator. The storage condition shall be properly documented. Both the receipt and dispensation of all test products (used and unused) are documented using forms provided by P&G or suitable forms provided by the site. Test products are returned to P&G following the trial, or alternatively, are destroyed at the clinical site with the prior written approval of P&G.

SUBJECT CONSENT

The Investigator obtains written informed consent for each subject prior to that subject's participation in the study, per the US Code of Federal Regulations, Title 21, Parts 50.25 and 50.27 and ICH-GCPs, Chapter 4, subpart 4.8. Subjects, or their legal guardian, are required to read, sign and date a consent form with the Investigator also maintaining a signed and dated copy. The subject or legal guardian will be given a copy of the consent form. All study procedures must be explained in non-technical terms.